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Original Article

Bacterial profile and their antibiotic resistance in an ICU of Bangladesh: Comparison of four studies from 2004 to 2011

Kaniz Fatema[1*], ASM Areef Ahsan[2*], Lovely Barai[3], Fatema Ahmed[4], Jalaluddin Ashraful Haq[5], Mohammad Omar Faruq[6]

Abstract

Antimicrobial resistance plays a vital role in determining the outcome of the critically ill patients with infections in intensive care unit (ICU).

Aim: *The objective of this study was to evaluate the frequencies of isolated pathogens and their resistance pattern in an ICU of Bangladesh. The study also aimed to analyze and compare the trends of bacterial population and their resistance pattern in the same ICU between 2004 and 2011 in four different studies.*

Materials and Method: *A cross sectional study was carried out in a 10 bed adult ICU of a tertiary care hospital of Bangladesh over a period of 10-month, from January 2011 to October 2011. Blood, respiratory secretions and urine from patients with clinically suspected infections were included in the study. Findings were compared with previous three studies done in 2004, 20006-07, and 2008-09 in same ICU.*

Results: *In 2011, a total of 1408 samples were analyzed. Six hundred eighty-four micro-organisms were isolated from 597 samples. Maximum growth was obtained from respiratory secretions (71.7%). Organisms isolated were Acinetobacter sp. (46.4%), Pseudomonas sp. (21.4%), Candida sp. (11.5%), Klebsiella sp. (9.9%), Eschericia coli (4.1%) and Staphylococcus aureus (2.9%). Major gram negative organisms were highly resistant to 3rd generation cephalosporins (>70%). All Acinetobacter sp. were extremely resistant (>85%) to all antibiotics except colistin. Resistance of isolated Pseudomonas sp. was >65% to ciprofloxacin, >70% to aminoglycosides, and >85% to imipenem. About 72% of the isolated Staphylococcus aureus was methicillin resistant with variable resistant to other antibiotics. Comparing the findings of this study with previous three studies done in same ICU, it has been found that rate of isolation of Acinetobacter sp., was increased significantly ($p<0.05$) in this ICU population with decrease in rate of Pseudomonas sp., Eschericia coli, Staphylococcus aureus, Enterococci sp., and Candida sp. Resistances pattern of two commonest gram negative organisms, eg Acinetobacter sp. and Pseudomonas sp., to the available antibiotics showed gradual increase in resistance from 2004 to 2011.*

Conclusion: *It is recommended that strict infection control policies and antibiotic stewardship program must be implemented to solve this emerging drug resistance problem which might cause high morbidity and mortality in ICU patients.*

Key Words: *Bacterial profile, Antimicrobial resistance, Intensive Care Unit*

Introduction:

Patients with serious infection are frequently admitted directly in the intensive care units (ICU), either from community or from hospital wards. Often, already admitted patients of ICU develop nosocomial ICU-acquired infection.[1] Infection with antibiotic resistant bacteria have become an increasingly difficult problem in ICUs leading to increased morbidity, mortality and costs. It has been reported by Centers for Disease Control and Prevention (CDC) in 2013 that more than two million people in USA were infected with resistant bacteria, with at least twenty-two thousand death among them in that year.[2] All antimicrobial resistance results from the convergence of two factors: poor infection control and selective pressure from antimicrobial agents.[3] Study results indicate that 30% to 60% of antibiotics prescribed in ICUs are unnecessary, inappropriate, or suboptimal.[4-6] Over-prescribing and misprescribing of antibiotics are directly related to emergence of resistant strains. A vicious circle of antibiotic use and resistance is thus created.

Despite the extensive knowledge of antimicrobial resistance, this problem has continued to emerge, especially in the ICUs. In an effort to reduce the development of antibiotic resistance, the recent guidelines by the Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS) recommended the regular generation of local antibiogram.[7] According to that recommendations, each hospital should develop and disseminate the antibiogram that is specific to the intensive care population of that hospital. The hospital antibiogram is a periodic summary of antimicrobial susceptibilities of local bacterial isolates submitted to the hospital's clinical microbiology laboratory.[8] Antibiograms are often used by clinicians to assess local susceptibility rates, as an aid in selecting initial empiric antibiotic therapy, and in monitoring resistance trends over time within an institution.

The aim of the present study was to determine the rate and pattern of organisms and their resistance pattern in an ICU of Dhaka, Bangladesh. As data on the changing pattern of organisms and their resistance to commonly used antibiotics are important for infection control activities in ICUs, comparison of this study (done in 2011) was done with previous three studies done in the same ICU by Basunia et al[9], Barai et al[10], and Fatema et al[11] in 2004, in 2006-07, and in 2008-09 respectively, to see the changing trends of antimicrobial resistance. This comparison is expected to make the intensivists and the microbiologists aware about the changing trends of microbes and their antimicrobial resistance. The knowledge of the antibiogram would also guide the intensivists to formulate the empiric therapy for the critically ill patients with infection.

Materials and Method:

This cross-sectional study was conducted in the 10-beded closed medical and surgical adult ICU of Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM). This is an academic hospital in the capital of Bangladesh. Samples from patients with clinically suspected infection who were admitted in this ICU from January 2011 to October 2011 were included in this study. Depending on the clinical suspicion about the source of infection, samples like blood, urine, and respiratory secretions (which included sputum, tracheal aspirate and broncho-alveolar lavage) were collected from the patients and sent to the department of Microbiology, BIRDEM. Standard microbiological methods were used to isolate bacteria from the specimens.[12] Antibiotic susceptibilities were done by Kirby-Bauer disc diffusion method.[13] Growth of three or more than three organisms in a single sample was considered as contaminated and so excluded from study.

Bacteriological profile and their antibiotic resistance pattern have been studied in same ICU previously in January to December, 2004[9], and from March 2006 to February 2007[10], and from October 2008 to March 2009.[11] The findings of 2011 was compared with the results of previous three studies done in the same ICU to determine the changing trends of organisms and their antibiotic resistance pattern.

1. Dr. Kaniz Fatema, Assistant Professor, Dept of Critical Care Medicine, BIRDEM General Hospital, Dhaka

2. Dr. ASM Areef Ahsan, Associate Professor and Head, Dept of Critical Care Medicine, BIRDEM General Hospital, Dhaka

3. Dr. Lovely Barai, Associate Professor and Head, Dept of Microbiology, BIRDEM General Hospital, Dhaka

4. Dr. Fatema Ahmed, Assistant Professor, Dept of Critical Care Medicine, BIRDEM General Hospital, Dhaka

5. Prof. Jalaludin Ashraful Haq, Professor of Microbiology, Ibrahim Medical College, Dhaka

6. Prof. Mohammad Omar Faruq, Professor, Critical Care Medicine, Ibn Sina Hospital, Dhaka

*First two authors have equal contribution and should be regarded as Principal Author

Corresponding Author:

Dr Kaniz Fatema
FCPS (Medicine), MD (CCM)
Assistant Professor
Dept of Critical Care Medicine (ICU complex, 6th floor)
BIRDEM General Hospital
122, Kazi Nazrul Islam Avenue
Dhaka 1000, Bangladesh
Email: drkanizfatemasb@gmail.com

Quantitative data has been expressed by percentage. SPSS 17 for windows has been used for statistical analysis. Comparison was done by Z-test of proportion and chi-square test. P value of <0.05 is considered significant.

Results:

A total of 1408 samples were sent to microbiology department from the ICU patients with suspected infection during the study period (January to October of 2011). Out of 1408 samples, growth was obtained from 597 samples (Table I). Some samples had growth of two organisms. Pattern of micro-organisms isolated from respiratory secretions, blood and urine has been shown in table II.

Table I: Rate of culture positivity and isolation rate of organisms in different samples

Sample type	Culture Positive Samples		Number of Isolates	
	Number	%	Number	%
Respiratory secretions (n=593)	418	70.4	491	71.7
Blood (n=576)	98	17	104	15.2
Urine (n=239)	81	33.9	89	13
Total (n=1408)	597	42.4	684	100

Table II: Pattern of organisms isolated from different samples

Organisms	Number isolated from			Total	
	Respiratory Secretion	Blood	Urine	Number	%
A. **Gram negative organisms**					
Acinetobacter sp.	288	26	4	318	46.5
Pseudomonas sp.	95	47	5	147	21.5
Klebsiella sp.	47	14	7	68	9.9
Eschericia coli	14	6	8	28	4.1
Proteus sp.	3	-	1	4	0.5
Flavobacterium sp.	3	1	-	4	0.5
Citrobacter sp.	2	-	1	3	0.4
Enterobacter sp.	1	-	-	1	0.1
B. **Gram positive organisms**					
Staphylococcus aureus	19	1	-	20	2.9
Staphylococcus epidermidis	-	-	1	1	0.1
Streptococcus pneumoniae	-	2	-	2	0.2
Enterococci sp.	-	-	2	2	0.2
Non-enterococci	-	2	5	7	1.1
C. **Fungi**					
Candida sp.	19	5	55	79	11.5
TOTAL	**491 (71.7%)**	**104 (15.2%)**	**89 (13.2%)**	**684**	

Among the isolated organisms, commonest organism was *Acinetobacter sp.*, followed by *Pseudomonas sp.*, *Candida sp.*, *Klebsiella sp.* and others (table II). The most common organism isolated in respiratory secretion was *Acinetobacter sp.* (58.6%). *Pseudomonas sp.* was the commonest isolated organism in blood (45.19%), and *Candida sp.* was the most frequent organism in urine (61.79%). Among the isolated *Candida sp.* in all samples, 49.37% were albicans and rests (50.63%) were non-albicans. Isolation frequency of gram negative organisms (83.7%) was significantly higher than the gram positive organisms (3.5%) and fungi (11.%) (83.7% vs 15%, $p<0.05$).

Antibiotic resistance of common gram negative organisms (*Acinetobacter sp., Pseudomonas sp., Klebsiella sp.* and *Eschericia coli*) have been shown in table III. About 28.5% of the isolated *Eschericia Coli* and 17.6% of isolated *Klebsiella sp.* were extended spectrum β-lactamase (ESBL) positive.

Table III: Antibiotic resistance pattern of major gram negative organisms

Antibiotics	*Acinetobacter sp.* n=318 x/y	*Pseudomonas sp.* n=147 x/y	*Klebsiella sp.* n=68 x/y	*Eschericia coli* n=28 x/y
Amikacin	316/318 (99.3%)	105/147 (71.4%)	30/58 (51.7%)	12/27 (44.4%)
Gentamicin	316/318 (99.3%)	129/147 (87.7%)	48/55 (87.2%)	17/27 (62.9%)
Netilmicin	272/309 (88.1%)	109/147 (74.1%)	43/60 (71.6%)	14/28 (50%)
Nitrofurantoin†	Not done	Not done	Not done	2/9 (22.2%)
Ceftriaxone	317/318 (99.6%)	133/147 (90.4%)	58/61 (95.08%)	27/27 (100%)
Ceftazidime	317/318 (99.6%)	107/147 (72.7%)	58/61 (95.08%)	26/27 (96.2%)
Cefotaxime	287/289 (99.3%)	130/147 (88.4%)	Not done	19/19 (100%)
Imipenem	303/318 (95.2%)	125/147 (85.03%)	34/62 (54.8%)	9/27 (33.3%)
Piperacillin+ Tazobactum	295/307 (96.09%)	31/135 (22.9%)	29/37 (78.3%)	9/14 (64.2%)
Colistin	6/338 (1.77%)	23/77 (29.8%)	0/32 (0%)	Not done
Ciprofloxacin	317/318 (99.6%)	98/146 (67.1%)	55/59 (93.2%)	27/28 (96.4%)
Cotrimoxazole	304/316 (96.2%)	104/147 (70.7%)	50/56 (89.2%)	ND
Chloramphenicol	Not done (57.4%)	27/47 (60%)	6/10	0/6

Resistance percentage is shown within parentheses

x =number of isolates resistant to particular antibiotic, y= total number of isolates tested for antibiotic susceptibility.

†Nitrofurantoin was tested only for *Eschericia coli* isolated from urine

Nineteen *Staphylococcus aureus* were isolated from respiratory secretions and only one from blood. Methicillin resistant *Staphylococcus aureus* (MRSA) was 72.3%. Though 50% of isolated *Staphylococcus aureus* were rifampicin resistant, none was resistant to vancomycin. Resistance to aminoglycosides and cotrimoxazole varied from 20% to 58%. Resistance to erythromycin was also high (94.7%).

A retrospective study was done in the same ICU in 2004.[9] Total 133 samples were sent at that time from the ICU patients. Out of 133 samples, 126 isolates were found and their antibiotic sensitivity were analyzed. In 2006 study, 1660 samples were analyzed and 564 samples yielded growth of 632 organisms in this ICU.[10] In another study done in 2008 in the same ICU, 211 samples were culture positive out of 536 samples, and there were 231 isolates.[11] Figure 1 demonstrated the percentage of samples which showed growth of micro-organisms. Comparison of these organisms in different years is shown in table IV, and pattern of major isolates in respiratory secretion, blood and urine in Figure 2, 3 and 4 respectively.

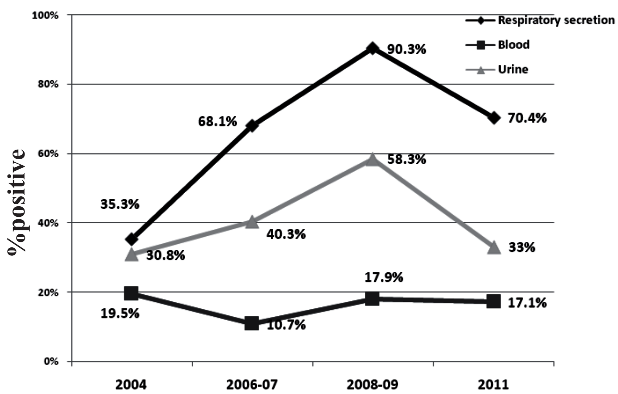

Fig 1: Comparison of culture positive rate of samples from present study (2011) with that of studies done in 2004, 2006-07, and 2008-09

Table IV: Comparison of frequency of major organisms isolated from different samples in different years

	2004 n=126	2006-07 n=632	2008-09 n=231	2011 n=684	*p* value
Pseudomonas sp	46 (36.5%)	184 (29.1%)	54 (23.3%)	147 (21.4%)	0.003
Acinetobacter sp	15 (11.9%)	174 (27.5%)	52 (22.5%)	318 (46.4%)	<0.001
Klebsiella sp	18 (14.2%)	61 (9.6%)	13 (5.6%)	68 (9.9%)	0.057
Eschericia coli	12 (9.5%)	65 (10.3%)	22 (9.5%)	28 (4.1%)	<0.005
Staphylococcus aureus	4 (3.1%)	9 (1.4%)	21 (9.1%)	20 (2.9%)	<0.001
Enterococci sp	5 (3.9%)	11 (1.7%)	10 (4.3%)	2 (0.2%)	<0.001
Candida sp	19 (15.1%)	81 (12.8%)	51 (22.1%)	79 (11.5%)	<0.005

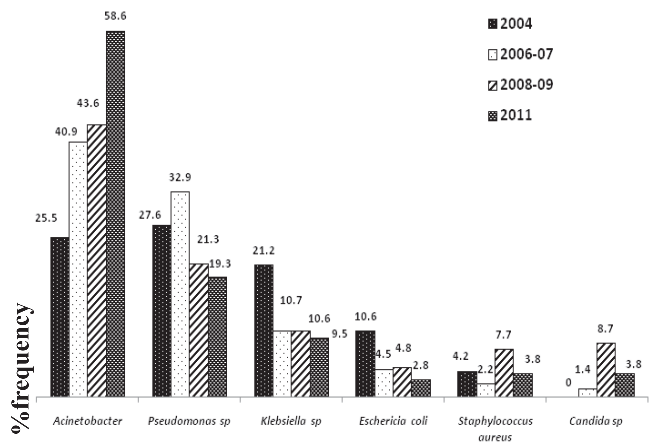

Figure 2: Pattern of major organisms isolated from respiratory secretions in all four studies

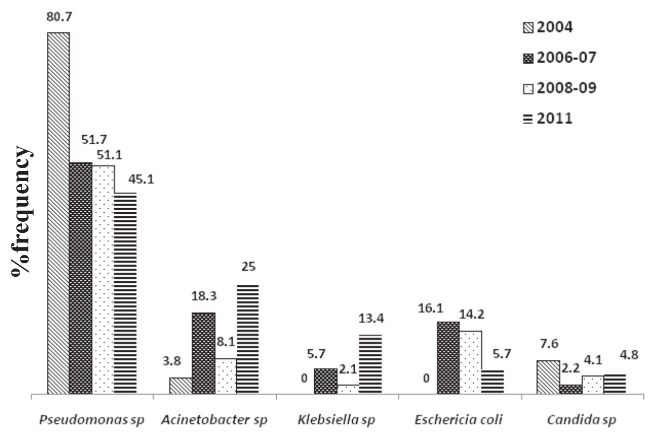

Figure 3: Pattern of major organisms isolated from blood in four studies

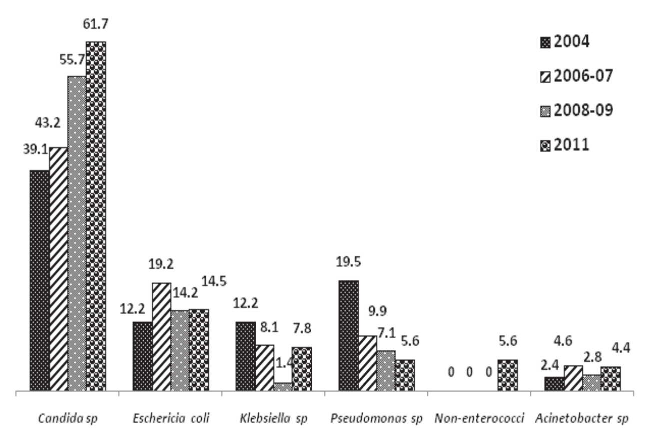

Figure 4: Pattern of major organisms isolated from urine in all four studies

Pseudomonas sp. and *Acinetobacter sp.* were the commonest organisms isolated from samples in the study ICU over these seven years. Pattern of resistance of isolated *Pseudomonas sp.* and *Acinetobacter sp.* to some specific antibiotics (which are usually used for treatment of that bacteria) are shown in the following tables (Table V and VI, respectively). As susceptibility of *Acinetobacter sp*. to colistin was not tested in past, so comparison could not be made.

Table V: Comparative resistance of isolated Pseudomonas sp. to selective antibiotics among the four studies (expressed in percentage)

	2004	2006-07	2008-09	2011	*p* value
Amikacin	100	81.4	90.7	99.3	>0.05
Gentamicin	91.7	93.2	89.5	99.3	>0.05
Netilmicin	61.5	77.9	75.4	88.1	<0.05
Ceftazidime	80	81.5	62.9	72.8	>0.05
Imipenem	33.3	72.4	66.1	95.2	<0.05

Table VI: Comparative resistance pattern of isolated Acinetobacter sp. to selective antibiotics among four studies (expressed in percentage)

	2004	2006-07	2008-09	2011	*p* value
Amikacin	70.4	62.5	88.4	71.4	>0.05
Gentamicin	91.7	93.2	88.6	99.3	>0.05
Netilmicin	61.5	77.9	67.3	88.1	<0.05
Ceftazidime	80	81.5	94.2	72.7	>0.05
Imipenem	38.1	57.6	74	85.1	<0.05

Discussion:

Antimicrobial resistance is an important determinant of outcome for patients in ICU. Rates of resistance have increased for most pathogens associated with infection among the ICU patients, especially hospital-acquired infections.[14] This results in extended hospital stay, increase in cost for treatment, and increase in morbidity and mortality. Regular microbiological surveillance should be done for implementation of better therapeutic strategies to reduce the high morbidity and mortality in the critical care setting. The present study was conducted as a part of continuous surveillance about the prevalence of isolated bacteria and their antimicrobial resistance pattern in the ICU patients at a tertiary care hospital in Bangladesh. As the pattern of organisms and their resistance pattern vary time to time, so the findings of the current study has been compared with previous three studies done in same ICU to see whether there was any change in the microbial isolates and their resistance pattern.

The study ICU had 6 beds in the year of 2004 which was increased to 10 beds in 2006. This may explain the increased number of culture positive samples and isolates found in the study by Barai et al, Fatema et al and this study than Basunia et al. Rate of isolation of organisms was higher in respiratory and blood samples in 2008-09 than other studies (Fig 1). Study in 2008-09 was done for six months which may explain the unusual number of positive samples in that study.

Maximum number of organisms was isolated from either tracheal aspirates or from sputum in the present study. This is probably due to the fact that most of the critically ill patients in ICU had respiratory problems and/or they were on mechanical ventilator. Similar findings were observed in ICUs of Denmark[15] and Jamaica[16] where most of the organisms were isolated from respiratory secretions (49% and 50%, respectively). Frequency of isolates in respiratory secretion and urine increased markedly from 2004 to 2008 (Fig 1). Numbers of respiratory and urinary isolates decreased in 2011 than 2008, but remain almost similar to findings of 2006. Frequency of organisms in blood samples remained almost similar from 2004 to 2011.

Vincent et al showed that the gram negative and gram positive bacteria were almost equal in frequency in a study done in 1417 ICUs of 17 countries of Europe.[17] But Shehabi et al found more gram negative organisms (49%) than gram positive (31%) in an ICU of Amman, Jordan.[18] We had the same finding. In our ICU, gram negative bacteria (84%) were the dominant isolated organism in all samples. Gram negative organisms were predominantly isolated also in ICUs of Greece and India (97% and 91.9%, respectively).[19,20]

Previously, *Pseudomonas sp.* was the commonest isolated organism in the study ICU.[9,10] But later, number of *Acinetobacter sp.* increased significantly (p<0.001) with subsequent decrease in the frequencies of other organisms like *Pseudomonas sp., Escherichia coli, Staphylococcus aureus, Enterococci sp.* and *Candida sp.*(Table IV). Number of isolated *Acinetobacter sp.* and *Pseudomonas sp.* was equal in a Turkish ICU.[21] But *Acinetobacter sp.* was the commonest organism in a Medical ICU of Ahmedabad, India (30.9%),[20] and also in an ICU of Greece (35%).[19] There was no significant change in number of isolated *Klebsiella sp.* over these seven years (from 2004 to 2011) in BIRDEM ICU.

Pattern of distribution of major organisms in respiratory secretions in figure 2 showed that the numbers of *Acinetobacter sp*. were increased gradually. Though *Pseudomonas sp.* was the commonest isolated organism in respiratory secretions in 2004, *Acinetobacter sp.* has replaced it from 2006 onwards. In blood, *Pseudomonas sp.* was the most common isolates from 2004 to 2011 (Fig 3). Number of *Acinetobacter sp.* and *Klebsiella sp*. increased in blood samples. *Candida sp.* held the top position among the organisms isolated from urine over these seven years (Fig 4). Among the bacterial isolates found in urine, *Pseudomonas sp.* was most common in 2004. But since 2006, *Eschericia coli* was the commonest bacteria in urine and its frequency remained more or less same from 2004 to 2011.

In our study 46.4% of the isolated organisms were *Acinetobacter sp.*, and 90.5% of isolated *Acinetobacter sp.* were found in respiratory samples. The prevalence of drug-resistant *Acinetobacter* strains is increasing, especially in ICU as admission in ICU is one of the important risk factor associated with infection by this gram negative cocco-bacilli.[22] Emergence of resistance occurs in the context of selective pressure from broad-spectrum antimicrobial therapy, especially carbapenems and 3rd generation cephalosporins.[23] Unfortunately treatment option is limited as most of the isolated strains were multi-drug resistant. The resistance of *Acinetobacter sp.* was >85% to aminoglycosides, and >90% to ciprofloxacin, 3rd generation cephalosporins and imipenem. They were sensitive only to colistin. The pattern of resistance of isolated *Acinetobacter sp.* to amikacin and ceftazidime was almost same from 2004 to 2011, but resistance to netilmicin and imipenem had increased significantly (p<0.05).

The second commonest isolated organism in this study was *Pseudomonas sp.* which occupied the leading position in past years. Though the number of isolated *Pseudomonas sp.* was more in respiratory secretion (64.62% of total isolated *Pseudomonas sp*.) than other samples, it was the most common isolated organism found in blood samples (45.1% of the blood isolates). The resistance of this notorious organism was >65% to ciprofloxacin, >70% to aminoglycosides and >80% to imipenem. Resistance to 3rd generation cephalosporins varied from 72 to 90%. Piperacillin and tazobactum combination was the sensitive antibiotic for these gram negative bacilli as resistance was less than 25%. Resistance of isolated *Pseudomonas sp.* to amikacin, gentamicin and ceftazidime does not vary much from 2004 to 2011. But resistance to netilmicin and imipenem has increased significantly (p <0.005 for both antibiotics).

Widespread use of extended spectrum cephalosporins, especially ceftazidime, has resulted in emergence of ESBL positive strains of *Enterobacteriaceae*.[3] Extensive use of oral trimethoprim/sulfamethoxazole and ciprofloxacin in community may also play role by facilitating spread of ESBL-producing organisms.[24] In Turkey, ESBL activity was found between 12% to 51% in ICUs of different hospitals.[21] ESBL producing *Eschericia coli* and *Klebsiella sp.* were also detected in our study, though in less number (28.5% and 17.6%, respectively). To combat this, inappropriate and unnecessary use of broad spectrum cephalosporins must be reduced.

In European ICUs, *Staphylococcus sp.* was the most frequently isolated organism (30.1%).[25] But in the study ICU, number of *Staphylococcus aureus* is always low (<10%). Similar finding was observed in Indian ICUs.[20] Unfortunately 72.3% of isolated *Staphylococcus aureus* in 2011, 50% in 2008-09, and 77% in 2006-07 were methicillin resistant. Carvalho et al found 60% MRSA in an adult ICU of Bazil.[26] As MRSA spreads through health-care personnels, enforcement of the infection control policies is the key to control MRSA.[3]

Candida sp. was the 2nd commonest organism in the study done by Basunia et al.[9] But Barai et al and we found it as the 3rd most frequent isolate.[10] Most of the *candida sp.* were found in urine sample. The current study was done in the ICU of BIRDEM, which is the largest hospital dealing with diabetic patients in Bangladesh. This immunocompromised state may be responsible for the growth of large number of yeast. Moreover, use of broad-spectrum antibiotics is also act as a risk factor for growth of *candida sp*.[16] Heavy use of azoles can cause change in epidemiology of candida species.[27] In our study we found 50.6% of *candida* were non-albicans. Usually yeasts found in urine and sputum or other respiratory secretions are usually due to colonization, and treatment is only needed in severely neutropenic or immunocompromised patients.[28] If blood sample shows growth of *candida sp.*, any intra-vascular device of more than 48 hour duration must be removed and treatment with anti-fungal should be started.[16,27] Before starting treatment with anti-fungals, testing for azole susceptibility is recommended for all blood-stream *Candida* isolates.[29]

We had some limitations. First, we did not calculate the statistical value of the changing trends of all microbes and their changing patterns. As there were different and multiple antibiotics, it was quite difficult to do statistical analyses of all bacteria. Rather, the visual inspection of the tables and graphs may give a better idea. Secondly, we have the major drawback of not testing for anti-fungal drug sensitivity in Bangladesh. Besides, samples like pus, wound swab, any drainage collection, CSF, pleural fluid and ascitic fluid were not included in the present study. Thirdly, we did comparison from 2004 to 2011. A current study to see the pattern of bacteria and their antimicrobial resistance pattern should be done.

Conclusion:

Worldwide, ICUs are faced with increasingly rapid emergence and spread of antibiotic-resistant bacteria.[30] Gradual increase in number of organisms and their resistance to available antibiotics was observed in this study. The commonly isolated gram negative organisms were highly resistant to ciprofloxacin and 3rd generation cephalosporins (>50%). Resistance to aminoglycosides and imipenem varied depending on type of organisms. With inappropriate antibiotic usage recognized as a key driver of resistance and few new drugs in pipeline, antibiotic stewardship program and infection control policies are the essential component of the overall strategy to reduce antibiotic resistance and better management of the critically ill patients. Otherwise, in near future, we will end up in pre-antibiotic era, when there will be no cure for simple infection, and people will die from minor ailment.

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